

FIG. 1A

Barnase coding sequence

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1 met ala gln val ile asn thr phe asp gly val ala asp tyr leu gln thr tyr
2 TCTAGACC ATG GCA CAG GTT ATC AAC ACG TTT GAC CGG GTT GCG GAT TAT CTT CAG ACA TAT
3 3'gttcacgagatctgg tac 5' (B1 primer)

1 his lys leu pro asp asn tyr ile thr lys ser glu ala gln ala leu gly trp
2 CAT AAG CTA CCT GAT AAT TAC ATT ACA AAA TCA GAA GCA CAA GCC CTC GGC TGG
3 (B4 primer) 3' t gtt cgg gag cgg acc5'

1 val ala ser lys gly asn leu ala asp val ala pro gly lys ser ile gly gly
2 GTG GCA TCA AAA GCG AAC CTT GCA GAC GTC GCT CCG GCG AAA AGC ATC GGC GGA
3 5'gca tca aaa ggg aac c 3' (B2 primer)

1 asp ile phe ser asn arg glu gly lys leu pro gly lys ser gly arg thr trp
2 GAC ATC TTC TCA AAC AGG GAA GGC AAA CTC CCG GGC AAA AGC CGA ACA TCG
3

1 arg glu ala asp ile asn tyr thr ser gly phe arg asn ser asp arg ile leu
2 CGT GAA GCG GAT ATT AAC TAT ACA TCA GGC TTC AGA AAT TCA GAC CCG ATT CTT
3

1 tyr ser ser asp trp leu ile tyr lys thr thr asp his tyr gln thr phe thr
2 TAC TCA AGC GAC TGG CTG ATT TAC AAA ACA ACA GAC CAT TAT CAG ACC TTT ACA
3

1 lys ile arg OCH
2 AAA ATC AGA taa
3

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FIG. 1B

Intergenic sequence

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CGAAAAAACGGCTTCCTGCGGAGCGCGTTTTTTTTCAGCTTTACATAAAGTGTGTAATAAATTTTCTTCAAACCTCTGATCGGTCAATTT
CACTTCCGGATCCGGTCCAATCTGCAGCGCTCCGAGACAGGAGACATCGTCCAGCTGAACCGGGCAGAAATCCGGCCATTCTCTGAAG
AGAAAAATGGTAAGTATAGATAAATCATAGAAAGAGCGGCAC

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FIG. 1C

Barstar coding sequence

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1 Met lys lys ala val ile asn gly glu gln ile arg ser ile ser asp leu his
2 ATG AAA AAA GCA GTC ATT AAC GCG GAA CAA ATC AGA AGT ATC AGC GAC CTC CAC
3
1 gln thr leu lys lys glu leu ala leu pro glu tyr tyr gly glu asn leu asp
2 CAG ACA TTG AAA AAG GAG CTT GCC CTT CCG GAA TAC TAC GGT GAA AAC CTG GAC
3
1 ala leu trp asp cys leu thr gly trp val glu tyr pro leu val leu glu trp
2 GCT TTA TCG GAT TGT CTG ACC GGA TCG GTG GAG TAC CCG CTC GTT TTG GAA TCG
3
1 arg gln phe glu gln ser lys gln leu thr glu asn gly ala glu ser val leu
2 ACG CAG TTT GAA CAA AGC AAG CAG CTG ACT GAA AAT GGC GCC GAG ACT GTG CTT
3
1 gln val phe arg glu ala lys ala glu gly cys asp ile thr ile ile leu ser
2 CAG GTT TTC CGT GAA GCG AAA GCG GAA GCG TGC GAC ATC ACC ATA CTT TCT
3
1 OCH
2 TAA TAGGATCAATGGAGATGAACAATATAGATCCCCCGGCTGCAGGAATTC
3 5'taa tacgatcaatggagatg 3' (B3 primer)

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- 1: Translation of DNA sequences encoding Barnase (A) and Barstar (C), respectively
- 2: DNA sequence encoding either Barnase (A), Barstar (C) or the synthetic intergenic region (B) according to Paul et al. (1992)
- 3: Sequence of DNA primers that were used for IPCR to construct pepA* (B3/B4) and pepB* (B1/B2).

FIG. 1D

Translational fusion of
ORF Peptide A**/ (Gly4 ser)3 Linker peptide / GUS

met ala gln val ile asp thr phe asp gly val ala asp tyr leu gln thr tyr his lva
tctagacc ATG GCA CAG GTT ATC AAC ACG TTT GAC GCG GTT GCG GAT TAT CTT CAG ACA TAT CAT AAG

leu pro asp asp tyr ile thr lva ser glu ala gln ala leu gly trp met gly gly gly
CTA CCT GAT AAT TAC ATT ACA AAA TCA GAA GCA CAA GCC CTC GGC TGG ATG GGC GGT GGC

gly ser gly gly gly ser gly gly gly ser gly ile pro gly tyr gly gln ser
GGT TCC GGT GGC GGT GGC AGC GGC GGC GGT GGT AGC GGC atc ccc ggg tac ggt cag tcc

pro met
ctt atg ... of GUS

Underlined: ORF of peptide A**

FIG. 1E

Nucleotide Sequence of Translational fusion of
Ubiquitin genomic sequence and ORF Peptide A***

tctagacc ATGCAGATCT TCGTGAAGAC CTTGACCGGC AAGACCATCA CTCTCGAGGT CGAGAGCAGC GACACCATCG
ACAATGTCAA GCGCAAGATC CAAGACAAAG AAGGTATCAT TCTTCTCAG TCAATCTCGA TCTTCTCTT TAGCTTTTIG
AAATTCAGAT CTCTTATCAT TTAATGTTT CTCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCGGAAA
GCAGCTCGAA GATGCCCGTA CTTGGCTGA CTACAACATC CAGAAAGGTA CGAATCCTTC TGTGATCAT
TTGATGATC TGATGTATA AACTCTAATG GATGTATC ATTGTAAC AGAATCTACA CTTTATCTTG TGTGAGGCT
TAGAGGTGGA GCACAGGTTA TCAACAGTT TACGGGGTT GCGATTATC TTCAGACATA TCATAAGCTA CCTGATAATT
ACATTACAA ATCAGAAGCA CAAGCCCTCG GCTGGATGTA Gaggatcc

Underlined: Introns A and B within the ubiquitin sequence.
Bold: glycine codon 76 at the end of the ubiquitin ORF

FIG. 1F

Nucleotide Sequence of Translational fusion of Ubiquitin genomic sequence and ORF Peptide B***

ctagacc ATGCAGATCT TCGTGAAC CTTGACCGG AAGACCATCA CTCTCGAGGT CGAGAGCAGC GACACCATCG
ACAATGTCAA GGCCAAAGATC CAAGACAAAG AAGTATATCAT TCTTCTCTAC TCAATCTGGA TTCTTCTCTT TAGCTTTTGG
AAATTCAGAT CTCTTATCAT TTAATTTTCTT CTTCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCCGAAA
GCAGCTCGAA GATGJCCGTA CTTTGGCTGA CTACAACATC CAGAAAGTGA CGAAATCATC CGAATCCCTT TGTGATCAT
TTGATGATC TGATGTGATA AACTCTAATG GATGTGATC ATTGTAAAC AGAATCTACA CTTTATCTTG TGTGAGGCT
TAGAGGTGGA GCATCAAAAG GGAACCTTGC AGAGCTCGCT CCGGGAAGA GCATCGCGG AGACATCTTC TCAAAACAGGG
AAGCAAACT CCGGGCAAA AGCGGACGAA CATGGCGTGA AGCGATATT AACTATACAT CAGCTTCAG AAATTCAGAC
CGAATCTTT ACTCAAGCGA CTGGCTGATT TACAACAA CCGACCATTA TCAGACCTTT ACAAAAATCA GATAA...

Underlined: Introns A and B within the ubiquitin sequence.
Bold: glycine codon 76 at the end of the ubiquitin ORF

FIG. 1G

DNA sequence of 1 PCR primers (example 1)

B5	5'	CACAAGTACTCTAGACCATG 3'	(forward)
B6	5'	CATCCAGCCGAGGGCTTGT 3'	(reverse)
B7	5'	GGCGGTGGCGTTCCG 3'	(forward)
B8	5'	CCACTAGTTCTAGAGTACTTGTG 3'	(reverse)
B9	5'	GCACAGGTTATCAACAG 3'	(forward)
B10	5'	GCGGATCCTCTACATCCAGCCGAGGGCTTGT 3'	(reverse)
B11	5'	GCATCAAAAGGGAACC 3'	(forward)
B12	5'	GGTCTAGAGTACTTGTG 3'	(reverse)
Ubq16F	5'	GCTCTAGACCATGCAGATCTTCGTGAAAC 3'	(forward)
Ubq1R	5'	CTGGATCCACCTCTAAGCCTCAACA 3'	(reverse)
Ubq1a	5'	TATGGATCCCCCGGCTGCAGGA 3'	(forward)
Ubq1b	5'	TCCACCTCTAAGCCTCAACAC 3'	(reverse)

FIG. 2
SCHEMATIC ILLUSTRATION OF pepA* AND pepB*
CONSTRUCTION BY INVERSE PCR (IPCR)

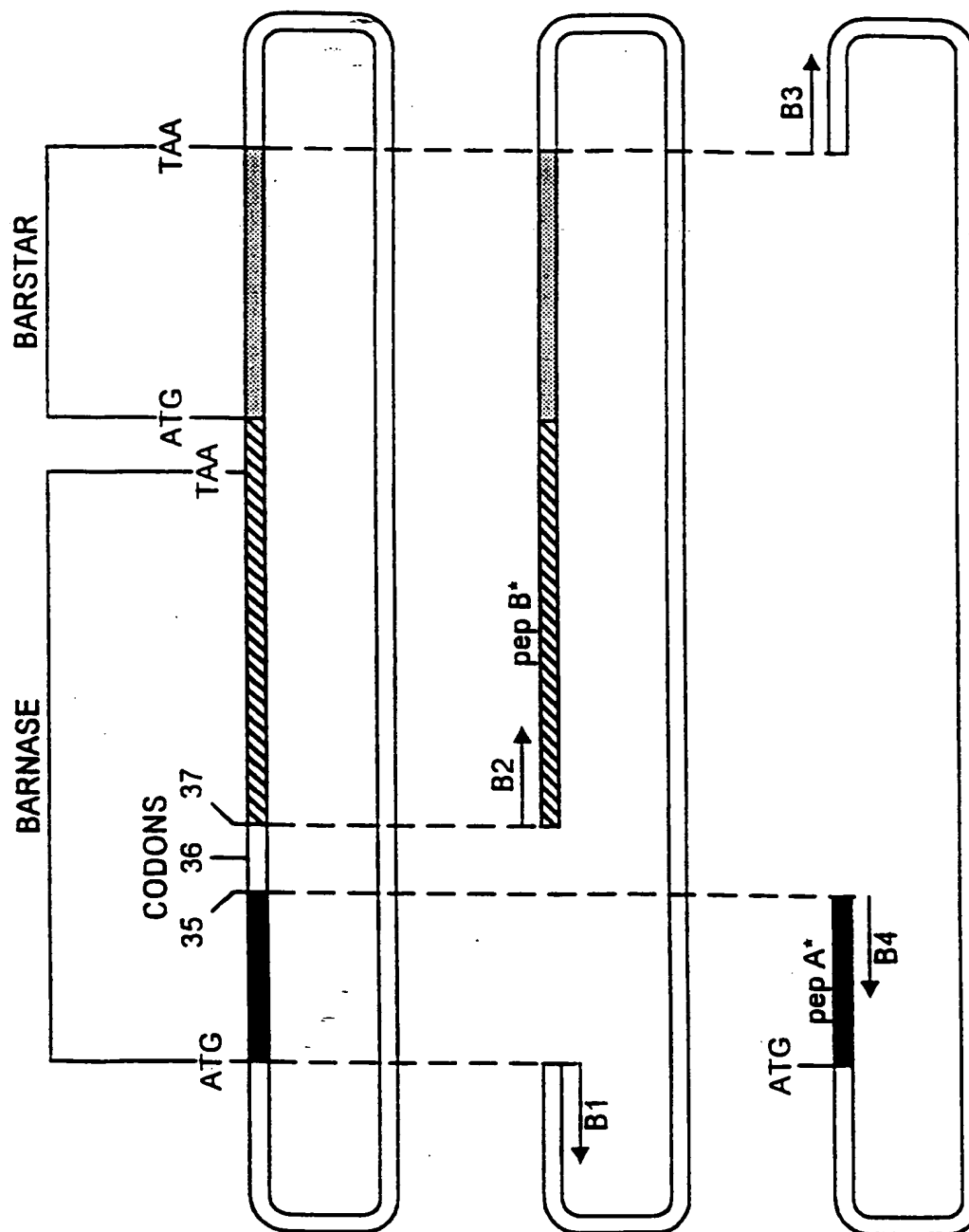


FIG. 3A

In Vitro Construction from Synthetic Oligonucleotides of S-peptide, S(+5)-protein and S-protein

1. 5'-gcggatccatgaaggagaccggcc-3OH
2. 5'-gcggatccatgaaggagaccggccggccaagtctcgagggccagcacatggacagc-3OH 5P-TAAAGATCTATG...
3. 3OH-GTACCTGTCTG_____ATTCTAGATAC-5'
4. 5'-ccagatcctatg----agctcctccaaactactg-3OH
5. ...AGACCTCCGCGCCAGCTCCTCCTCCAACTACTGCAACCAAGATGAAGTCT-3OH 5P-AGGAACCTGA...
6. 3OH-ACTACTTCAGA_____TCCTTGGACT-5'
7. ...CCAAGGACAGGTGCAAGCCAGTCAACACCTTCTGCCAGAGAGCCCTGGC-3OH 5P-CGATGTCCAG...
8. 3OH-CTCGGACCG_____GCTACAGGTC-5'
9. ...GCCGTCTGCAGCCAGAAGACGTGGCCTGCAAGAACGG-3OH 5P-TCAGACCAACT...
10. 3OH-CGTCTTGCC_____AGTCTGGTTGA-5'
11. ...GCTACCAGTCTACAGCACCATGTCCATCACCAGTGCCTGCGGAGACCGG-3OH 5P-CTCCAGCAAG...
12. 3OH-GCTCTGGCC_____GAGTCTGTTTC-5'
13. ...TACCCTAACTGCGCTACAGACCAACCCAGGCCCAACAAGCACATC-3OH 5P-ATTGTTGCCCTG...
14. 3OH-GTTCGTGTAG_____TAACAACGGAC-5'
15. 3OH-CTGCGGAGGCAGATTtccctagggc-5'
16. ...CGAGGGTAACCCCTTACGTGCCTGTCTCCACTTCGACGCCCTCCGTCTAAaggatcccg-3OH

FIG. 3B In Vitro Construction from Synthetic Oligonucleotides of the Sequence encoding the S-peptide and the (Gly4-Ser)3 Linker

1. 5'-gcggatccATGAAGGAGACCGCC-3OH
2. 5'-gcggatccATGAAGGAGACCGCCGCCCAAGTTCGAGCGCCAGCACATGGACAGC-3OH
3. 5P-GGCGGTGG...
3OH-GTACCTGTCTG_____CCGCCACC-5'
4. ...CGGTTCCGGTGGCGGTGGCAGCGCGCGGGTGGTAGCaagatcttcggg-3OH
5. 3OH-CCATCGTctctagaagccc-5'

Protein and DNA Sequences of S-peptide and S-peptide with (Gly4 Ser)3 linker

Legend to Figure 4 A:

- 1: DNA sequence of the synthetic Bovine RNase A gene (codon 1 to 15) according to N. Vasantha and David Filpula (1989)
- 2: Translation of synthetic DNA sequences encoding Bovine RNase A
- 3: DNA sequence of the S-peptide coding sequence referred to in this invention
- 4: DNA sequence encoding the S-peptide with (gly4 ser)3 linker peptide referred to in this invention

FIG. 4B

Protein and DNA Sequences of S(+5)-protein and S-protein

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1  --- ACC ACC AGT GCT GCC AGT TCT TCC AAC CAG ATG ATG AAG TCT AGA AAC TTG ACC ACC AAG
2  met ser thr ser ala ala ser ser asn tyr cys asn gln met met lys ser arg asn leu thr lys
3  agatct atg AGC ACC tcc gcc GCC agc tcc TCC AAC TAC tgc AAC CAG ATG ATG AAG TCT agg AAC ctg ACC AAG
4  agatct atg --- --- --- agc tcc TCC AAC TAC tgc AAC CAG ATG ATG AAG TCT agg AAC ctg ACC AAG

1  GAC AGA TGT AAG CCA GGT AAC ACA TTT GTC CAC GAG AGT TTG GCT GAT GTC CAA GCC GTC TGC AGT
2  asp arg cys lys pro val asn thr phe val his glu ser leu ala asp val gln ala val cys ser
3  GAC agg tgc AAG CCA gtc AAC acc ttc GTC CAC GAG agc ctg gcc GAT GTC cag GCC GTC TGC agc
4  GAC agg tgc AAG CCA gtc AAC acc tcc GTC CAC GAG agc ctg gcc GAT GTC cag GCC GTC TGC agc

1  CAG AAA AAC GTT GCA TGC AAG AAC GGT CAA ACG AAC TGT TAC CAG AGT TAC AGC ACC ATG TCC ATC
2  gln lys asn val ala cys lys asn gly thr asn cys tyr gln ser tyr ser thr met ser ile
3  CAG aag AAC gtc gcc TGC AAG AAC GGT cag acc AAC tgc TAC CAG tcc TAC agc ACC ATG TCC ATC
4  CAG aag AAC gtc gcc TGC AAG AAC GGT cag acc AAC tgc TAC CAG tcc TAC agc ACC ATG TCC ATC

1  ACT GAC TGT CGT GAG ACA GGC TCG AGC AAG TAT CCT AAT TGT GCT TAC AAG ACC ACA CAG GCG AAC
2  thr asp cys arg glu thr gly ser ser lys tyr pro asn cys ala tyr lys thr thr gln ala asn
3  acc GAC tgc cgc GAG acc GGC tcc AGC AAG tac CCT aac tgc gcc TAC AAG ACC acc CAG gcc AAC
4  acc GAC tgc cgc GAG acc GGC tcc AGC AAG tac CCT aac tgc gcc TAC AAG ACC acc CAG gcc AAC

1  AAA CAC ATC ATT GTT GCT TGT GAA GGT AAC CCT TAC GTT CCT GTC CAC TTT GAC GCC AGT GTT TAA
2  lys his ile ile val ala cys gly glu gly asn pro tyr val pro val his phe asp ala ser val OCII
3  aag CAC ATC ATT GTT gcc tgc gag GGT AAC CCT AAC CCT TAC gtg CCT GTC CAC ttc GAC GCC tcc gtc TAA
4  aag CAC ATC ATT GTT gcc tgc gag GGT AAC CCT TAC gtg CCT GTC CAC ttc GAC GCC tcc gtc TAA

1  ---
2  ---
3  aggatcc
4  aggatcc

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Legend to Figure 4 B:

- 1: DNA sequence of the synthetic Rhase A gene (codons 16 to 124) according to Vasanthia and Filpula (1989)
- 2: Translation of DNA sequences encoding the Bovine RNase A
- 3: DNA sequence of the synthetic S(+5)-protein coding sequence (aa16 to aa124)
- 4: DNA sequence of the synthetic S-protein coding sequence (aa21 to aa124)

FIG. 4C

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i. PCR amplification product encoding impartial AOX3 targeting signal

XbaI / BglII

tctagatcttaac ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG
TGGGCGGAGC TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA
GACACCAGCA CGGTGGCGGC GCGTTTGGAA GCTTCCA ctttaagcggatcc
AflII / BamHI

ii. ORF encoding AOX3 targeting sequence (underlined) and S-peptide

ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AAGGAGACCG CCGCCGCCAA GTTCGAGCGC
CAGCACATGG ACAGCTAA

iii. ORF encoding AOX3 targeting sequence (underlined) and S-peptide-(Gly4 Ser)3-GUS

ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AAGGAGACCG CCGCCGCCAA GTTCGAGCGC
CAGCACATGG ACAGCGGCGG TGGCGGTTCC GGTGGCGGTG GCAGCGGCGG CGGTGGTAGC
GGGATCCCCG GGTACGGTCA GTCCCTTATG --> GUS

iv. ORF encoding AOX3 targeting sequence (underlined) and S-protein

ATGAAGAATG TTTTAGTAAG GTCAGCTGCG CGAGCTCTGC TTGGCGGCGG TGGGCGGAGC
TACTACCGCC AGCTCTCAAC GGCGGCGATC GTGGAACAGA GACACCAGCA CGGTGGCGGC
GCGTTTGGAA GCTTCCACTT AAGAAGGATG AGCTCCTCCA ACTACTGCAA CCAGATGATG
AAGTCTAGGA ACCTGACCAA GGACAGGTGC AAGCCAGTCA ACACCTCCGT CCACGAGAGC
CTGGCCGATG TCCAGGCCGT CTGCAGCCAG AAGAACGTGG CCTGCAAGAA CGGTCAGACC
AACTGCTACC AGTCCTACAG CACCATGTCC ATCACCAGCT GCCGCGAGAC CGGCTCCAGC
AAGTACCCTA ACTGCGCCTA CAAGACCACA CAGGCCAACA AGCACATCAT TGTTCCTGTC
GAGGGTAACC CTTACGTGCC TGTCCACTTC GACGCCTCCG TCTAA

v. Translational fusion of Ubiquitin genomic sequence and ORF of S-protein

ATGCAGATCT TCGTGAAAAC CTTGACCGGC AAGACCATCA CTCTCGAGGT CGAGAGCAGC
GACACCATCG ACAATGTCAA GGCCAAGATC CAAGACAAAG AAGGTATCAT TCTTCCTCAG
TCAATCTGGA TTCTTCTCTT TAGCTTTTGT AAATTCAGAT CTCTTATCAT TTACTTGTTT
CTCCTTTAAG GAATCCCTCC GGATCAGCAG AGATTGATCT TCGCCGGAAA GCAGCTCGAA
GATGGCCGTA CTTTGGCTGA CTACAACATC CAGAAAGGTA CGAAATCATC CGAATCCTTC
TGTTGATCAT TTCGATGATC TGATTGTATA AAGTCTAATG GATTGTTATC ATTTGTAAC
AGAATCTACA CTTTATCTTG TGTGAGGCT TAGAGGtGg tCagCTCCA ACTACTGCAA
CCAGATGATG AAGTCTAGGA ACCTGACCAA GGACAGGTGC AAGCCAGTCA ACACCTCCGT
CCACGAGAGC CTGGCCGATG TCCAGGCCGT CTGCAGCCAG AAGAACGTGG CCTGCAAGAA
CGGTCAGACC AACTGCTACC AGTCCTACAG CACCATGTCC ATCACCAGCT GCCGCGAGAC
CGGCTCCAGC AAGTACCCTA ACTGCGCCTA CAAGACCACA CAGGCCAACA AGCACATCAT
TGTTCCTGTC GAGGGTAACC CTTACGTGCC TGTCCACTTC GACGCCTCCG TCTAA

Underlined: introns A and B within the ubiquitin encoding sequence

Bold: codon for Glycine⁷⁶, marking the C-terminus of the ubiquitin.

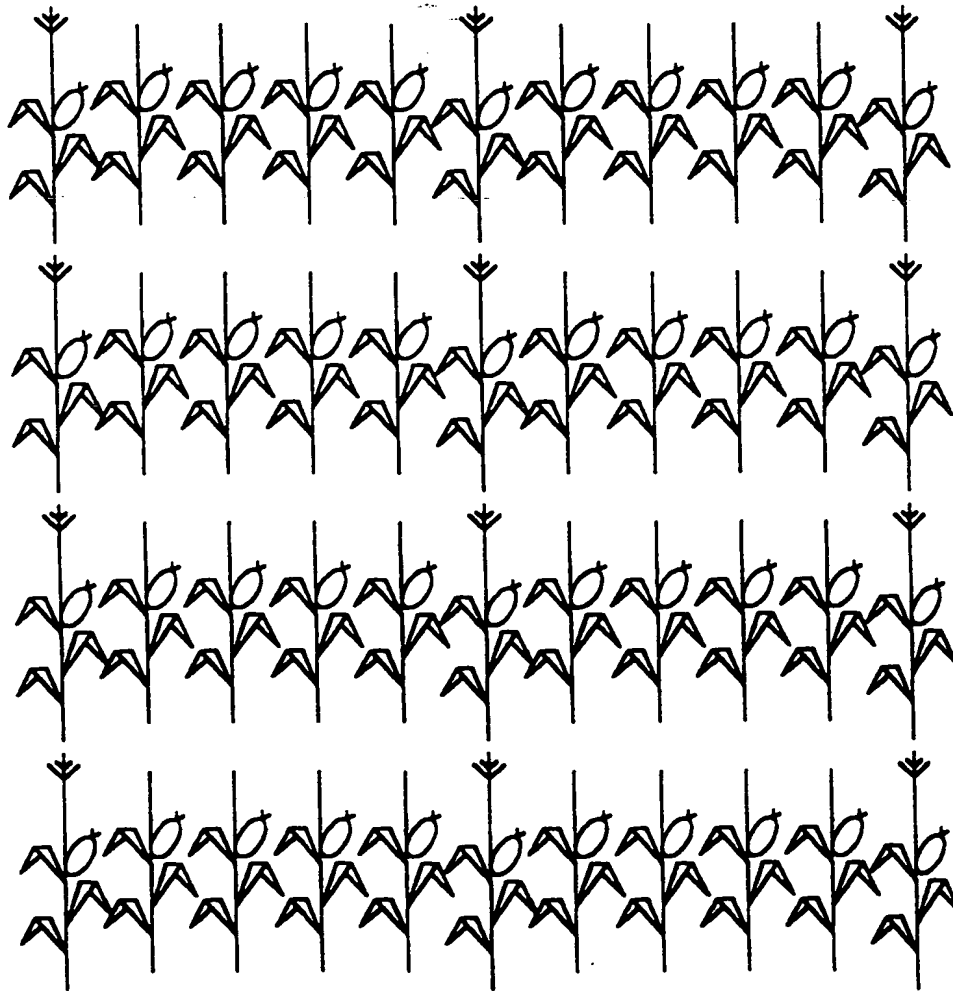
Small letters: PCR introduced conservative codon changes to generate a BamHI site and to modify the codon usage

FIG. 4D

Nucleotide sequence of T PCR primers (example 3)

Sprot F	5'	GGTGGATCCAGCTCCAACTACTGCAAC	3'
Sprot R	5'	CGGGATCCTTAGACGGAGGCGTCG	3'
SprotMI1	5'	GTCCTTAAGAAGGATGAGCTCCTCCAACTAC	3'
SprotMI2	5'	CGGGATCCTTAGACGGAGGCGTCG	3'
SpepMI1	5'	GTCCTTAAGAAGGATGAAGGAGACCGCCG	3'
SpepMI2	5'	TCGGGATCCTTAGCTGTCCATGTGCTG	3'
SpepGMI2	5'	TCGGGATCCTCATTGTTTGCCTCCCTG	3'
AOX3MI1	5'	TGCTCTAGATCTTAACATGAAGAATGTTTTAG	3'
AOX3MI2	5'	TCGGATCCGCTTAAGTGGAAGCTTCCAAAC	3'

FIGURE SHOWING A PRODUCTION SCHEME OF EMBRYO LESS MAIZE GRAINS:
LINES A AND B ARE SOWN IN ALTERNATIVE ROWS (FOR EXAMPLE ONE MALE
AND FOUR FEMALES)



LEGEND
(REFER TO
DESCRIPTION
FOR DETAILS)



MALE PARENT A



FEMALE PARENT B

FIG. 5